Effects of Acute Sublethal Levels of pH on the Feeding Behavior of Juvenile Fathead Minnows

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Abstract
This study was conducted to determine the impact of acute sublethal pH levels on the feeding behavior of juvenile fathead minnows. Eighteen to 24 day-old juveniles were fed live or dead brine shrimp under light or dark conditions in order to identify the role of the senses of vision, chemoreception, and mechanoreception in feeding at different pH's. Feeding trials were conducted at various pH combinations; 5.0, 7.0, 10.0; 4.5, 7.0, 11.0; and 3.5, 7.0, 11.5. Total mortality was observed at pH 3.5 and 11.5. Appetitive behavior was present at all pH levels as evidenced by frequencies of occurrence of feeding ranging from 93-100%. No relationship was observed between pH and the number of fish feeding. The fathead minnow is chiefly a visual feeder and vision was not affected at any pH level as 99.9% of all brine shrimp, live and dead, were consumed in the light. Significantly fewer brine shrimp were consumed in the dark than in the light and significantly fewer brine shrimp were consumed in the dark at the lower pH's than in the dark at pH's 7.0+. No measurable impact on feeding behavior was observed at pH 7.0 and 10.0+. Chemoreception was stressed at low pH levels. The ability of chemoreception and mechanoreception to successfully function in consort in capturing living prey in the dark at low pH levels was noticeably impacted. The effect of low pH on mechanoreception was not determined.

Keywords: pH, fathead minnow, feeding behavior

Introduction

The science of behavioral toxicology is a recently developed diagnostic approach to measuring and recording observations of behavior that reflect biochemical and ecological responses of organisms to environmental contamination (Little 1990).

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Behavioral activities are rapidly becoming recognized as highly sensitive indicators of sublethal toxicity (Diamond et al. 1990, Little and Finger 1990). A variety of behaviors has been used to study sublethal toxicities including ventilation and cough frequencies, feeding activities, temperature preference, predator avoidance, swimming performance, schooling behavior, and pH detection and avoidance (Hill 1989). However, while it is readily acknowledged by investigators that differing behavior activities involve a diversity of sensory-motor pathways and physiological processes (Sandheinrich and Atchison 1990), little attention has been given to the impacts that toxicants selectively impart to specific senses or sensory pathways. Although the

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embryo-larval-juvenile life cycle stages are accepted as being the most sensitive for toxicity tests (McKim 1977), little consideration has been given to the impacts of sublethal toxicants upon the sensory systems of these life cycle stages, many of which exhibit a gradient of sense organ development from the time of hatching until the completion of successful behavior formation (Noakes and Godin 1988).

Although acid stress and depressed pH conditions have been studied at length from many different perspectives (Zischke et al. 1983, Leino et al. 1987, Mills et al. 1987, Jansen and Gee 1988, among others), and are receiving much local press in relation to acid precipitation, little attention has been given to the effects of acid stress on fish behavior (Jones et al. 1985). Lemly and Smith (1985) summarized the literature supporting fathead minnows as being among the most acid sensitive fishes. Jones et al. (1985), in pursuing the effects of sublethal pH levels on the behavior of arctic char, reported acid stress to suppress chemoreception. Lemly and Smith (1985, 1987) found acidification to significantly affect the ability of fathead minnows to detect or respond to chemical stimuli. Jones et al. (1985) described this chemo-suppression to likely result from the reduced stimulatory nature of amino acids at reduced pH's and the damage of epithelial tissues (olfactory epithelium) by acidic conditions. Lemly and Smith (1987) suggested that increased olfactory mucous thickness in response to lowered pH prevented normal stimulus-receptor interaction and/or that chemical interaction at the subcellular level was impaired because of stearic/charge changes at the receptor cells. Whatever the explanation, these observations are of potentially profound importance to environmental biologists in that they represent avenues for unrecognized massive larval mortalities among those fish species that are dependent upon chemoreceptors of chemoreceptors/mechanoreceptors in the formation of exogenous feeding behavior.

The purpose of this study was to determine the effects of different acute pH levels on the senses of vision, mechanoreception and chemoreception in the feeding behavior of juvenile fathead minnows, <u>Pimephales promelas.</u>

Methods and Materials

<u>Test Fish</u>

Fathead minnows used in the project were obtained within 12 hours of hatching from the U.S. EPA Newtown Fish facility, Newtown, Ohio, on 3 July, 1990. Fish were maintained in 2 1 finger bowl in ASTM water at 24+/- 1C and were fed freshly hatched brine shrimp twice daily, 0800 and 1700 h. At the onset of the pH trials, the minnows were 18 days old and averaged 11.58 mm in total length (range 10.1 mm - 13.0 mm). All experimentation was conducted at the Graduate Center for Toxicology at the University of Kentucky, Lexington, KY.

<u>pH Test Solutions</u>

pH solutions in 3.0 1 aliquots were prepared using ASTM water with Nitric acid to produce low pH levels and Sodium Hydroxide to produce high pH levels. A pH of 7.0 was achieved by adding either Nitric acid or Sodium Hydroxide as required. An Orion pH meter was used to determine pH levels in producing the desired pH concentrations. Acid pH's tested were 5.0, 4.5, 4.0, and 3.5. Basic pH's were 10, 10.5, 11.0, and 11.5. An acidic, a basic, and a pH 7.0 concentration, was used daily for four consecutive days in the following sequence: Day 1 - pH's 5.0, 7.0, 10.0; Day 2 - pH's 4.5, 7.0, 10.5; Day 3 - pH's 4.0, 7.0, 11.0; Day 4 - pH's 3.5, 7.0, 11.5.

<u>Sense Organ Isolation</u>

Each daily combination of pH test solutions was applied to four different feeding regimes, living brine shrimp fed in the light, living brine shrimp fed in the dark, dead brine shrimp fed in the light, and dead brine shrimp fed in the dark (see Table 1 for design).

Table 1. Frequency of occurrence of juvenile fathead minnows eating at least one brine shrimp during live-dead, light-dark, feeding trials at varying pH levels.

pH Level	5.0	7.0	10.0
Light-Live	5/5 5/5 4/5	5/5 5/5 4/5	5/5 4/5 4/5
Light-Dead	3/5 5/5 5/5	5/5 5/5 5/5	5/5 5/5 5/5
Dark-Live	4/5 5/5 5/5	5/5 5/5 5/5	5/5 5/5 5/5
Dark-Dead	5/5 5/5 5/5	5/5 5/5 3/3	5/5 5/5 5/5
	(93%)	(98%)	(95%)
pH Level	**4.5**	**7.0**	**10.5**
Light-Live	5/5 5/5 5/6	5/5 4/5 5/5	5/5 5/5 5/5
Light-Dead	5/5 4/5 4/5	5/5 5/5 5/5	5/5 5/5 5/5
Dark-Live	5/5 5/5 5/5	5/5 5/5 5/5	5/5 5/5 5/5
Dark-Dead	5/5 5/5 5/5	5/5 5/5 4/5	5/5 5/5 5/5
	(95%)	(95%)	(100%)
pH Level	**4.0**	**7.0**	**11.0**
Light-Live	5/5 5/5 5/5	5/5 5/5 5/5	5/5 5/5 5/5
Light-Dead	5/5 5/5 5/5	4/5 5/5 5/5	5/5 5/5 5/5
Dark-Live	5/5 5/5 5/5	5/5 5/5 5/5	4/5 4/5 5/5
Dark-Dead	5/5 5/5 4/4	6/6 5/5 5/5	4/4 4/4 4/4
	(100%)	(98%)	(96%)
pH Level	**3.5**	**7.0**	**11.5**
Light-Live	Total	No	Total
Light-Dead			
Dark-Live	Mortality	Test	Mortality
Dark-Dead			

<u>Test Procedure</u>

A total of 180 minnows were selected at approximately 1600 h the day before a trial. The fish were separated into 12 groups of 15, each group of which was placed in a 500 ml beaker containing water of a specific pH concentration (daily test combinations described above). The fish were then placed in an environmental chamber at 25.0 C with an 8 hour dark period (2200 to 0600 h) for acclimation until approximately 1300 h the following day. Five fish from each pH concentration were then placed in each of three 150 ml finger bowls containing fresh mixtures of the test pH's for replicate trials. The fish were allowed to acclimate in the finger bowls for ten minutes in either light or dark before food was added. Light feeding trials with live and dead brine shrimp were conducted prior to similar dark feeding trials.

<u>Feeding</u>

Brine shrimp (Salt Lake City variety) were raised in the laboratory and fed immediately following 24 hours incubation. The brine shrimp used in the feeding trials were the same variety and size used to raise and maintain the minnows. Average brine shrimp length was 0.7 mm. Fifty live or dead brine shrimp for each fish subsample were selected

Table 2. Number and Percent of brine shrimp remaining following juvenile fathead minnow live-dead, light-dark feeding trials at varying pH levels.

pH Level		5.0			7.0			10.0	
Light-Live	0	0	0	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	4	0	0 (2.7%)	0	0	0	5	5	3 (8.7%)
Dark-Dead	9	14	8 (20.7%)	8	3	0 (7.3%)	2	0	0 (1.3%)
		(5.8%)			(1.8%)			(2.5%)	
pH Level		4.5			7.0			10.5	
Light-Live	0	1	2	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	17	22	23 (41.3%)	1	1	3 (3.3%)	0	0	0
Dark-Dead	15	5	21 (27.3%)	9	0	0 (6.0%)	2	0	0 (1.3%)
		(17.2%)			(2.3%)			(0.3%)	
pH Level		4.0			7.0			11.0	
Light-Live	0	0	0	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	30	8	36 (49.3%)	0	0	0	2	0	0 (1.3%)
Dark-Dead	1	3	7 (7.3%)	1	0	1 (1.3%)	3	2	2 (4.7%)
		(14.2%)			(0.3%)			(1.5%)	
pH Level		3.5			7.5			11.5	
Light-Live		Total			No			Total	
Light-Dead									
Dark-Live		Mortality			Test			Mortality	
Dark-Dead									

with a 10 cc syringe and counted using a dissecting microscope. Each of nine syringes was loaded immediately prior to the feeding exercise and the fifty brine shrimp added to each group of five fish following the ten minutes acclimation to the test pH's. Feeding time for all tests was ten minutes. Brine shrimp for the dead feeding trials were killed by treatment in an ultrasonic bath for two to four minutes. Fish were aspirated from the test dishes immediately following the feeding trial and isolated in holding dishes. While the number of brine shrimp ingested by each individual fish could not be determined, the number of fish having consumed at least one brine shrimp was recorded using a dissecting microscope. Each feeding test dish was examined with the aid of a dissecting microscope and the number of brine shrimp remaining following the feeding trial was counted. All fish used in a feeding exercise were excluded from further feeding.

Results

Survival

All fish survived every trial except those at pH 3.5 and 11.5 in which 100 percent mortality was observed.

Frequency of Occurrence of Feeding

The number of fish ingesting at least one brine shrimp during the feeding trials ranged from 93 to 100 percent for all pH levels (Table 1). No relationship was observed between number of fish feeding and light or dark or live or dead food conditions. No relationship between frequency of occurrence of fish feeding and decreasing or increasing pH levels was observed. The average frequency of occurrence of feeding by fishes in pH 7.0 water, 97.7%, was the same as that (97.7%) for fish in the increasing pH level trials and only slightly greater than that (96.1%) for fish in the decreasing pH level trials (Table 1).

Light-Dark Feeding versus pH Level

With the exception of three brine shrimp at pH 4.5, all food organisms (99.9%), live or dead, were consumed during light feeding trials, at all pH levels (Table 2). In the dark, however, live or dead brine shrimp remained after feeding in 15 of 18 trials. The number of brine shrimp remaining in each dark trial ranged from 1.3% to 49.3% (Table 2). At pH 7.0, slightly more dead brine shrimp remained in the daŕk than live brine shrimp, 5,0% and 1.1%, respectively. At the lower pH's 5.0, 4.5, and 4.0 combined, more live brine shrimp (31.1%) remained in the dark than dead shrimp (18.4%). The number of live and dead brine shrimp remaining at the higher pH's was generally similar to that of pH 7.0 (Table 2). Significantly fewer live brine shrimp were consumed at pH 4.5 and 4.0 than at the higher levels while significantly fewer dead brine shrimp were consumed at pH 5.0 and 4.5 (Table 2).

Discussion

Based upon the high frequency of occurrence of feeding (93+%) by juvenile fathead minnows at all pH levels during all test regimens, appetitive behavior was concluded to be present to the lethal pH levels of 3.5 and 11.5. Hill (1989), in a chronic study of low pH effects on feeding behavior of smallmouth bass, also observed no loss of appetitive behavior at pH 4.2. Mortality at pH 3.5 in this study was consistent with the report by Mount (1973) that most lethal pH values recorded from laboratory data occur below 4.0. No similar data were found regarding high pH mortality, although Carlender (1969) reported the fathead minnow to have a broad tolerance for pH. The persistence of appetitive behavior through all pH levels was further supported by the minnows eating all except three (99.9%) live and dead brine shrimp fed during the light feeding trials. Consequently, since appetitive behavior persisted throughout the study, any observed reductions in feeding activity were considered to be the result of the selective impairment of sense organs by the different pH levels, or the behavioral inactivation of the feeding response during certain environmental conditions of the test (i.e.,dark), or possibly a combination of both these features.

According to the feeding patterns observed in this study, the fathead minnow is predominantly a visual, daylight feeder. The removal of all except three brine shrimp, live and dead, in the light at all pH levels identified the eyes, or the eyes in conjunction with the senses of chemoreception and mechanoreception, as the major sense organs involved in early life stage feeding. Klemm (1985) reported the fathead minnow to be primarily omnivorous and to possess large black eyes,presumably functional if heavily pigmented, at the time of hatching. The eye must play a strategically greater role in early life stage feeding as indicated by the recommendations by Birch et al. (1975) and Klemm (1985) that fathead minnow fry at least 6 days to 28 days old be fed live, freshly hatched (small) brine shrimp during the day, while older individuals may be fed frozen brine shrimp or varying forms of dry chow. This recommendation suggested a greater visual feeding success on living prey by larval-juvenile individuals and more successful visual-chemoreceptive feeding behavior in older individuals.

The eyes did not appear to be functionally impacted by different pH levels in this study. All brine shrimp, living and dead except for three individuals, were consumed in the light at all pH levels. However, the small test chambers (150 ml) with concomitant short reaction distance and number of brine shrimp per trial (50) might have alleviated any eye stress that was present or developing. Hill (1989) reported low pH levels to impair visual acuity, coordination, and agility, subsequently resulting in lower growth in chronic trials with smallmouth bass. The question of acute versus chronic study interpretations is brought into focus at this point. Hill's (1989) suggestion that acute bioassays may be too short to detect certain biological parameters, such as growth and survival, is not contested. However, in meeting the objectives of studies such as this, acute sublethal tests, especially feeding, may be more sensitive than chronic growth studies (Sandheinrich and Atchison 1990). That the fathead minnow is not an adept nocturnal feeder was supported by the number of uneaten live and dead brine shrimp in dark feeding trials at all pH's. The presence of more dead (5.0%) than live (1.1%) brine shrimp following dark feeding trials at pH 7.0 indicated a slightly greater ability or behavioral preference by the fathead minnow to select living food over dead food in the dark. However, at the lower pH's during the dark, feeding was greatly reduced and more dead brine shrimp were consumed than live shrimp.

Chemoreception was considered to be impaired at pH 5.0 when 20.7% of the dead brine shrimp remained, worsened at pH 4.5 when 27.3% remained, and then inexplicably improved at pH 4.0 when 7.3% of the shrimp remained. The marked improvement in dead feeding at the lowest pH could not be explained. Feeding on dead food in the dark was considered to be entirely a function of chemoreception since stimuli for visual and mechanoreceptive senses were not present. Consequently, since appetitive behavior was known to exist, this decreased feeding on dead food in the dark strongly suggested chemoreceptive inhibition. Yoshii and Kurihara (1983) reported that bluegill without functional lateral lines did not produce successful feeding strikes in the dark based on chemoreception alone. The omnivorous feeding capability of certain species such as bluegill sunfish and fathead minnow might employ the sense of chemoreception at levels not yet described and different than other species. However, the findings by Jones et al. (1985) that pH 5.0 suppressed chemo-orientation in the Arctic char, and Lemly and Smith (1985, 1987) that pH 6.0 eliminated fathead minnow responses to chemical stimuli supported the initial conclusions drawn in this study that chemoreception was impaired by the lower pH levels.

Living prey in the dark seemed to represent the maximum sensory challenge in feeding, especially for the visual feeding fathead minnow. Consequently, low pH exhibited its greatest impact on feeding on live brine shrimp in the dark. Although live brine shrimp were successfully preyed upon in the dark at pH 7.0 and higher, only slightly more than 50% were captured at the lowest pH's. Live food under dark conditions would suggest the involvement of the combined senses of chemoreception and mechanoreception in successful feeding. Enger et al. (1989) and Montgomery (1989) presented evidence substantiating the role of mechanoreceptors in detecting moving prey. Montgomery (1989) further described the role of mechanoreceptors as operating synergistically with vision in daylight planktivory and singly in total darkness. No mention was made by Montgomery of any receptor involvement with mechanoreceptors in dark feeding. Hara

(1986) summarized the extensive literature reviews on the role of chemoreception in feeding behavior and identified the first step in the feeding sequence as arousal to the presence of food which is primarily mediated by olfaction. Atema (1980) excepted the most visual fish species, i.e., anosmic sticklebacks, from the above behavior and suggested that accompanying senses such as mechanoreception and vision may also be involved in initial prey recognition. Should these sensory assumptions be correct and chemoreception was impaired to a reduced level of effectiveness in establishing the presence of living food, then several explanations regarding the role of mechanoreception in dark feeding become likely. First, mechanoreceptors act synergistically with chemoreception in dark-live feeding and the impairment of one sense automatically reduced the success of the other; secondly, mechanoreceptors also were impaired by the lowered pH rendering them incapable of successfully detecting and/or locating the moving prey; or thirdly, mechanoreceptors alone are incapable of successfully detecting and locating living prey in complete darkness in strongly visually directed species.

Summary
Fathead minnow feeding behavior was not observed to be affected by any non-lethal pH in the light. The interaction of vision with chemoreception, or mechanoreception, or both, produced successful feeding on live brine shrimp at all pH levels. Likewise, in the dark at pH's of 7.0 and higher, the senses of chemoreception and mechanoreception operated successfully in feeding on live food (98.9%) in the dark. However, at low pH's in the dark, chemoreception and mechanoreception were impaired in detecting and locating living prey. While chemoreception was observed to be stressed at low pH's, no evidence of such an effect on mechanoreception was detected. Future studies using streptomycin sulfate (Montgomery 1989) or cobalt (Karlsen and Sand 1987) to ablate mechanoreceptors might provide valuable insights into the role of mechanoreceptors in initiating as well as concluding the feeding response.

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